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CD4+ T-Lymphocytopenia in Long-Term Survivors Following Intensive Chemotherapy in Childhood Cancers

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Background. It is generally believed the effects of short intensive courses of therapy are rapidly reversible in childhood cancers, and immunologic function following years of maintenance treatment with chemotherapy usually returns to normal by 6 months or less when treatment is terminated. However, we previously demonstrated that dysregulation of immunoglobulins, especially IgD, was observed in long-term survivors following intensive chemotherapy in cancer patients. With regard to cellular immunity, investigators reported that antineoplastic chemotherapy significantly reduces the number of CD4+ T-lymphocytes, and production of newly developing CD4+ T-lymphocytes was inversely related to the patients' age. However, the incidence of CD4+ lymphocytopenia in long-term survivors of childhood cancers is not known.

Procedure. Here, we report the flow cytometric analysis of peripheral blood from long-term survivors who continue complete remission off chemotherapy for more than 5 years.

Results. Six out of 74 long-term survivors (8.1%), showed low CD4+ T-lymphocyte count (<300/mm^3). Three of six patients showed continued CD4+ T-lymphocytopenia over a year. In spite of the persistent low levels of CD4+ T cells, these three patients were not susceptible to severe infections.

Comment. Intriguingly, in patients with CD4+ T-lymphocytopenia there has been a tendency toward increased numbers of natural killer cells or γδ T cells that may be operating as a thymus-independent compensatory mechanism to defend the hosts. Med. Pediatr. Oncol. 30:40–45, 1998. © 1998 Wiley-Liss, Inc.

Key words: CD4+ T-lymphocytopenia; long-term survivors; childhood cancers

INTRODUCTION

CD4+ lymphocytopenia occurs in a broad spectrum of diseases, infectious and noninfectious [1]: (1) various pathogens including retrovirus, adenovirus, herpesviruses, parvoviruses, papilomaviruses, measles virus, Hepatitis B virus, Rocky Mountain and Mediterranean spotted fever, histoplasmosis, cryptococcosis and coccidiomycosis, leishmaniasis, tuberculosis, and brucellosis; (2) autoimmune disorders; (3) malnutrition; (4) congenital disorders including partial adenosine deaminase deficiency and partial DiGiorge syndrome; (5) drugs: corticosteroid and others; (6) lymphoproliferative disorders including thymoma and intestinal lymphangiectasia; and (7) old age or pregnancy. In addition, some reports have documented abnormalities in T-cell number and function after bone marrow transplantation and antineoplastic chemotherapy [2–4]. These abnormalities contribute to morbidity and mortality via infectious complications in patients. However, incidence of CD4+ lymphocytopenia in long-term survivors of childhood cancers has not been reported.

We previously reported that dysregulation of IgD was observed in 31 of 82 long-term survivors (37.8%) in childhood cancers [5]. Here, we show that CD4+ T-lymphocytopenia occurs in some long-term survivors who underwent intensive chemotherapy for hematological malignancies.

PATIENTS AND METHODS

Patients

To study late sequelae of intensive chemotherapy in childhood cancers, immunological studies were done in 1994 and 1995. In the study of 1994, these were 90 long-term survivors (children, M/F = 45/45) who continue complete remission off chemotherapy for more than 5 years. These long-term survivors have been followed up once a year. Average age at presentation, at the time of study, and average follow-up duration after chemotherapy were 5.2 yr, 15.4 yr, and 10.2 yr (S.D. = 3.8, 4.7, and 4.0), respectively. Of these, 16 patients were excluded from this study. Exclusion criteria in this study included patients who received hemopoietic stem cell

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transplantation, either autologous or allogeneic, patients who received thymic irradiation, and patients with documented infections. The rest of 74 children had malignancies including 36 acute lymphoblastic leukemias (ALL), 6 acute myelocytic leukemias, 7 non-Hodgkin's lymphomas (NHL), 11 neuroblastomas, and 14 miscellaneous solid tumors. The diagnosis of leukemias was based on the French-American-British (FAB) criteria [6]. The diagnosis of solid tumors was based on the pathological specimen using Hematoxylin-Eosin and other staining methods. Chemotherapy for ALL was previously described in detail elsewhere [7]. Briefly, the patients received vincristine-steroid-based chemotherapy followed by 24 Gy cranial irradiation. In addition to chemotherapy, they received immunotherapy that consisted of irradiated leukemic cells with biological response modifiers (BRMs), including BCG or streptococcal product OK432 [7]. Treatment duration chemoimmunotherapy was 3 yr and immunotherapy alone for another 2 yr. Patients with NHL received sequential cycles of vincristine, steroid, high-dose methotrexate, cyclophosphamide, VP-16, cytosine arabinoside, adriamycin, and cranial irradiation (18 Gy) for remission induction, followed by maintenance. Treatment duration of NHL is 3.0 yr. Solid tumors were treated with vincristine, cyclophosphamide, anthracyclines, VP-16, actinomycin D, and so on with or without carboplatin. The treatment results of hematological malignancies and solid tumors are competitive to the published reports (unpublished data).

Immunologic Analysis

Flow cytometric analysis was done as previously described [8]. Monoclonal antibodies for CD3, CD4, CD8, CD16/56, CD19, T cell receptor-αβ, and -γδ (Becton-Dickinson, Mountainview, CA) were used. Peripheral blood from patients were analyzed on FACScan flow cytometer (Becton-Dickinson) after appropriate processing. Recently, the U.S. Centers of Disease Control's working definition for idiopathic $CD4^+$ T-lymphocytopenia (ICL) should formally exclude several other conditions known to decrease $CD4^+$ T cells and persistent rather transient $CD4^+$ lymphocytopenia should be required (i.e., at least two counts more than 6 months apart) [9]. Thus, peripheral blood from the same patients were analyzed in 1994 and 1995, at least 6 months apart. Functional studies of lymphocytes were not done in the current series. IgG, IgA, IgM, and IgD were measured by serum protein electrophoresis and immunelectrophoresis. IgE was measured by a radioimmunosorbent test. All patients appeared clinically well at the time of blood collection.

Statistical Considerations

Significant differences between values obtained in each assay were determined by Fisher's exact probability test and simple regression analysis, using StatView 4.0 software on Macintosh Quadra950 computer. The difference was considered significant when $P < 0.05$.

RESULTS

Prevalence of $CD4^+$ T-Lymphocytopenia

Figure 1 demonstrates that six out of 74 patients (8.1%) developed low $CD4^+$ T lymphocyte counts (<300/mm^3) in a 1994 study (Fig. 1). A year later, we repeated the surface marker analysis in the same group of 74 patients. Three out of six patients showed again low $CD4^+$ T lymphocyte counts (Fig. 1). The rest of 71 children showed $CD4^+$ T lymphocyte equal to or more than 300/mm^3 in the second analysis. We do not have precise immunological data at onset of diseases in the year of 1979–1980. Moreover, since long-term survivors visit our hospital only once a year, we do not know the short-term results. None of these patients were susceptible to opportunistic infections.

Surface Marker Analysis and Immunoglobulins

Flow cytometric analysis of these three patients done in 1994 was shown in Figure 2 (patients 1, 2, and 3). Absolute $CD4^+$ T cell counts in the analysis in 1994 and 1995 were also shown in Figure 2. Table I represents the immunologic characterizations of these three children (2 ALL and 1 NHL). All these patients were less than 6 yr of age at onset. Reversed CD4/CD8 ratios were found in all three patients (0.38, 0.65, and 0.52, respectively). Thus, these three children had continuously low $CD4^+$ T cell count during the observation period. These three children did not show mediastinal involvement by radiographic imaging. As for chemotherapeutic drugs affecting $CD4^+$ T cell count, patients 1 (ALL, Down syndrome) and 2 (ALL) received dexamethasone for remission induction and maintenance therapy. They did not receive cyclophosphamide. Patient 3 (NHL, stage IV) received cyclophosphamide (cumulative dosis of 14.4 g per square meter of body surface area). With regard to infectious episodes, patient 2 complained of occasional bacterial diarrhea and showed hyper-IgD (16.7–41.5 mg/dL; normal range = 0.1–10.4 mg/dL). Patients 1 and 3 did not have increased frequency of infections. Interestingly, patient 3 had a relatively high percentage of natural killer (NK) cells that may have functioned in host defense to microorganisms. Informed consent to test retrovirus infections could not be obtained in these patients. Although they did not receive human immunodeficiency virus (HIV) tests, it is unlikely that they were infected with HIV since they did not belong to a high-risk group of HIV infection and clinical evidence of immunodeficiency develops within 10 yr of seroconversion in most subjects infected with HIV [10].

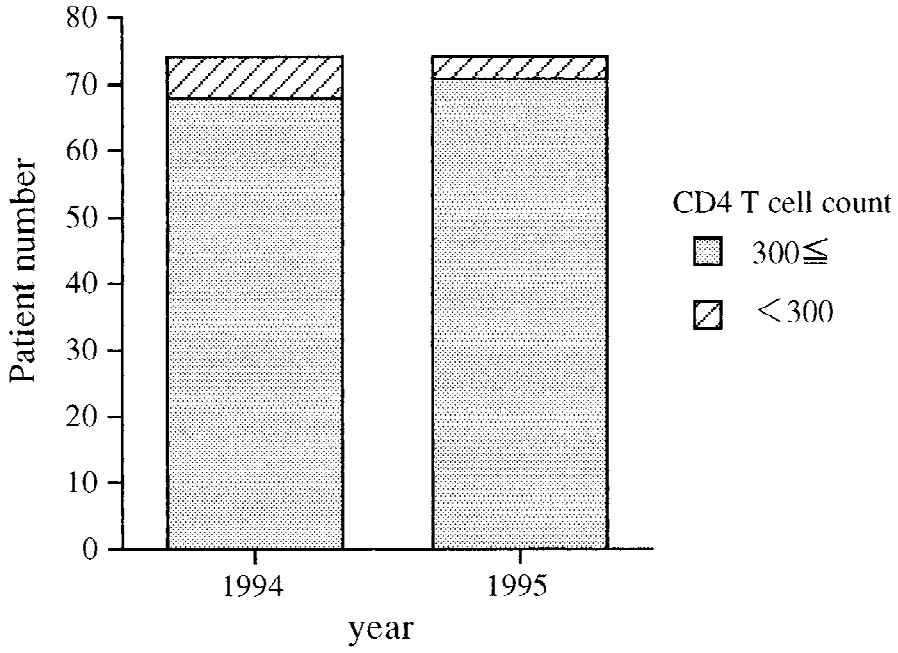

Fig. 1. Prevalence of $CD4^+$ T-lymphocytopenia in long-term survivors. Flow cytometric analysis revealed that $CD4^+$ T-lymphocytopenia ($CD4^+$ T cell <300/mm^3) was found in six out of 74 patients (8.1%) in 1994 and in three out of the same 74 patients (4.1%) in 1995.

Correlation With Other Cell Types

A total of 74 patients was analyzed in the combination of CD4 versus other cell types in 1994. Correlation between $CD4^+$ T cell count versus NK cell count and $CD4^+$ T cell count versus γδT cell count was depicted in scattergrams (Fig. 3). As shown in Figure 3A, B, there was a tendency of inverse correlation between $CD4^+$ T cells versus NK cells ($r = -0.23$) and $CD4^+$ T cell versus γδT cell ($r = -0.18$), although statistically not significant ($P = 0.08$ and 0.09 by simple regression analysis, respectively).

DISCUSSION

In this study, we have demonstrated that some children develop $CD4^+$ T-lymphocytopenia a long time after the cessation of antineoplastic chemotherapy. There are several reports that chemotherapy affects $CD4^+$ T cell counts. Mackall et al. [4] showed that high-dose sequential chemotherapy, given to relatively young patients without bone marrow involvement from tumor, consistently induces severe lymphocyte depletion. The mean value for $CD4^+$ T cells during chemotherapy in their study was 105/mm^3. Brunvand et al. [2] reported that two women with Stage II breast carcinoma treated with lumpectomy followed by breast irradiation and adjuvant chemotherapy developed Pneumocystis carinii pneumonia while receiving cytotoxic chemotherapy. Two women had profound lymphopenia, reversed CD4/CD8 ratios, and normal peripheral blood total leukocyte counts at the time of their infections. The patients' CD4 lymphocyte counts increased after chemotherapy for breast carcinoma was discontinued. Thus, Brunvand et al. [2] concluded that the therapy they received may have caused severe T-lymphocyte-mediated immunosuppression. From these reports it is suggested that acute $CD4^+$ T-lymphocytopenia occurs in some cancer patients after intensive chemotherapy and that it may be related to opportunistic infections. However, the prevalence of $CD4^+$ T-lymphocytopenia in long-term survivors following intensive cancer chemotherapy has not been reported.

In the present study, three out of 74 long-term survivors showed $CD4^+$ T-lymphocytopenia in two separate occasions. One out of three patients had hyper-IgD. We previously reported that hyperimmunoglobulinemia D (hyper-IgD) was observed in 31 of 82 children who were in complete remission off chemotherapy with the median follow-up duration of 4.5 yr after cessation of chemotherapy, suggesting that dysregulation of IgD synthesis persists a long time after cessation of antineoplastic drugs [5]. Likewise, we speculate that other cell types, including $CD4^+$ T-lymphocytopenia, may be a late sequella of intensive cancer chemotherapy. We have demonstrated that approximately 4% of long-term survivors after antineoplastic chemotherapy had low $CD4^+$ T-lymphocytopenia. However, idiopathic $CD4^+$ T-lymphocytopenia (ICL) is also a possible diagnosis in our patients. ICL, as defined by absolute $CD4^+$ counts <300 cells/mm^3 and / or <20%, has been reported in HIV-negative patients [9]. We cannot exclude the possi-

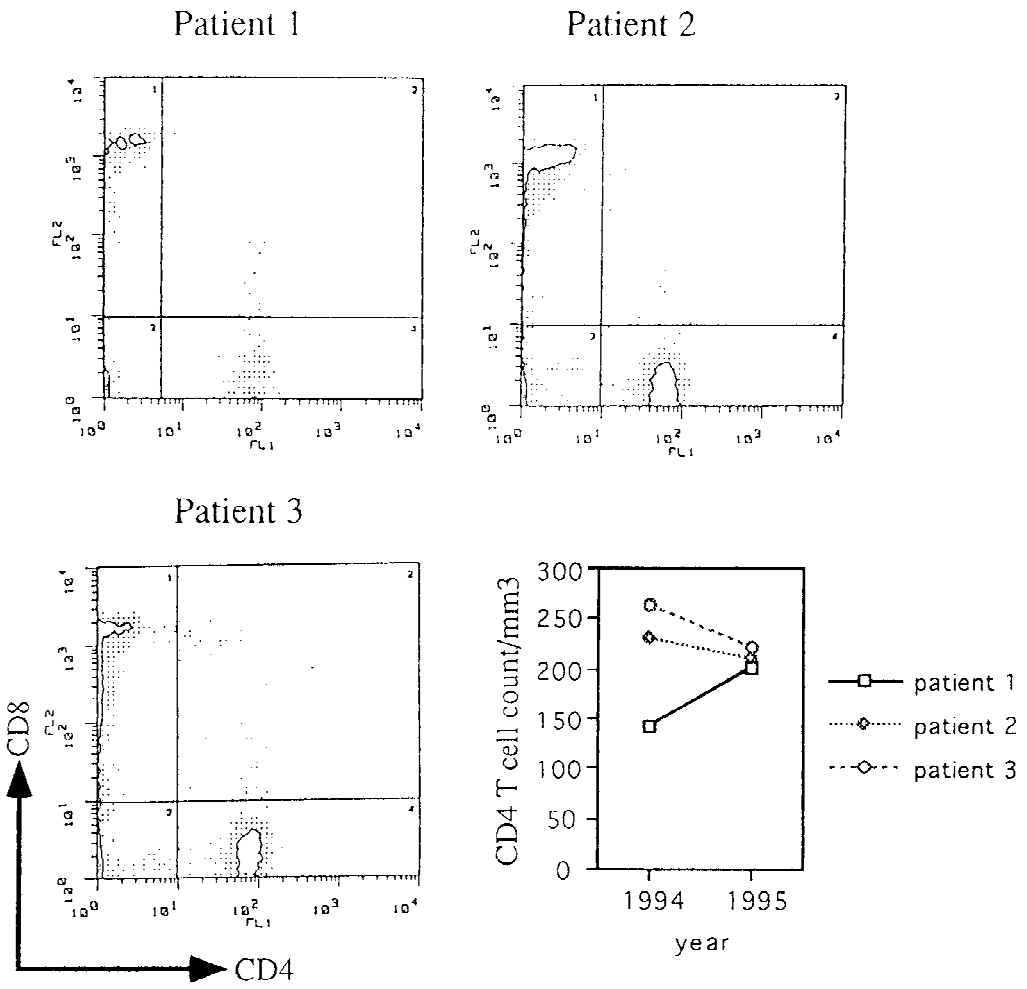

Fig. 2. Flow cytometric analysis of three patients with CD4+ T-lymphocytopenia. Patients 1, 2, and 3 showed a low percentage of CD4+ T cell and absolute count. Changes in absolute count of CD4+ T cells are also shown in the figure.

bility of ICL in the current study. However, the incidence of CD4+ T-lymphocytopenia is much higher in the present study than that of ICL. In the screening of blood donors for ICL, Busch et al. [9] reported that five out of 2,030 donors (0.25%) were found to have ICL [9]. In our study, three out of 74 patients (4.1%) had CD4+ T-lymphocytopenia ($P = 0.0003$ by Fisher's exact probability test). However, it cannot be excluded that the incidence in the study population of long-term survivors (4.1%) is enriched in comparison to the control population of the literature (0.25%) due to the fact that the risk of developing a malignancy is higher in children with immunodeficiencies. We cannot also exclude that possibility of CD4+ lymphocytopenia caused by other diseases as discussed in the Introduction. However, patients in the current study appear to be free from those diseases from the clinical point of view.

Although a close correlation between the duration of immunosuppression and abnormal CD4+ T cell levels has been suggested, its relationship to immunosuppressive agents is often difficult to assess. The agents used to achieve and maintain complete remission (prednisone, cyclophosphamide, etc.) are directed at modifying T cell function and lymphokine production. Cyclophosphamide is known to be immunosuppressive; it may have a more dramatic effect on lymphocyte populations than other agents. Conversely, other chemotherapeutic regimens might induce much less immunotoxicity. It has been reported that chemotherapy is immunosuppressive, but such suppression is almost always acute and not prolonged [11]. In the present study, BRMs were administered in ALL patients (patients 1 and 2). However, the role of BRM in abnormal CD4+ T cell levels may be minimal in light of the fact that other patients (patient 3) treated with antineoplastic drugs alone also showed CD4+ T-lymphocytopenia.

Mackall et al. [4] showed that thymic production of CD4+ T-lymphocytes was observed in cancer patients without thymus and bone marrow involvement under age 18 yr. Our results suggest that thymus-dependent regeneration of CD4+ T-lymphocytes may not occur in some small children (less than age 6 yr; patients 1, 2, and 3)

TABLE I. Immunologic Characterization of Three Patients With CD4⁺ T-lymphocytopenia*

Pt no.	Disease	Sex	Age at onset (yr)	Present age (yr)	Elapsed time (yr)	abs 1y[a] count/ mm³	T cell (%)	CD4 (%)	CD4 count/ mm³	CD8 (%)	CD4/ CD8 ratio	γδ T cell (%)	NK cell (%)	B cell (%)	IgG (mg/ dl)	IgA (mg/ dl)	IgM (mg/ dl)	IgD (mg/ dl)	IgE (IU/ ml)
1	ALL, Down synd	M	1.3	15.7	14.4	2450	24.4	5.8	142	15.3	0.38	11.9	3.1	2.3	1921	219	91	5.3	27
2	ALL	M	5.3	21.6	16.2	972	61.9	23.7	230	36.2	0.65	8.3	13.2	12.2	1103	160	165	16.7	54
3	NHL, stage IV	M	3.3	18.9	15.5	1364	38.2	19.3	263	36.9	0.52	10.5	27.5	20.4	1084	293	286	1.8	129

*All patients showed reversed CD4/CD8 ratio. Patient 3 had increased NK cells.
[a]abs 1y count: absolute lymphocyte count.

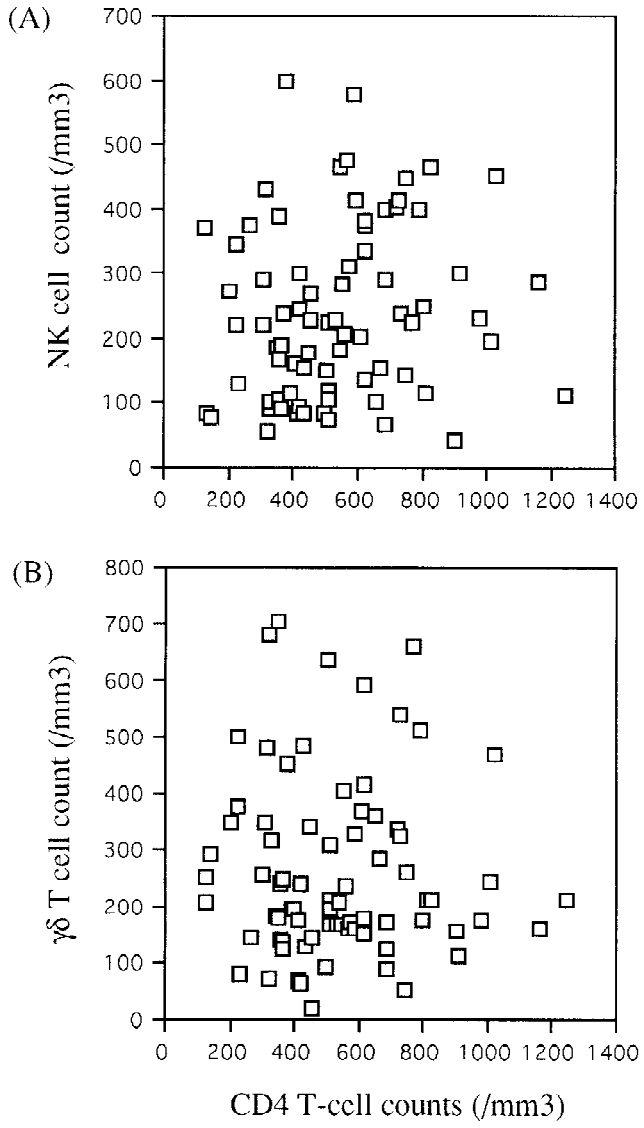

Fig. 3. Correlation between CD4⁺ T cells versus NK cells and CD4⁺ T cells versus γδ T cells in 74 long-term survivors. Although statistically not significant, there is a tendency for CD4⁺ T cell count to be inversely correlated with NK cells **(A)** or γδT cells **(B)** that may be functioning as a thymus-independent compensatory mechanism to defend the hosts.

with hematological malignancies involving bone marrow. Despite continued CD4⁺ T-lymphocytopenia, the overall incidence and severity of infections in our patients appear to be less than in patients with similarly decreased CD4⁺ counts in other circumstances (i.e., HIV infection, postbone marrow transplantation). This suggests that other factors may play a role in defending cancer patients from overwhelming infection. Possibilities include NK cells and γδ T cells that appear to be relatively spared with the regimens used in this report and which have been shown to play a role in the host response to fungal infection (Fig. 3). Human γδ T cells are an important component of the human fetal immune

system [12]. Postnatally, γδ T cells are involved in the host defense against various infections [13,14]. In spite of these data, we cannot fully explain why these patients do not suffer from infections in the observation period. Although we speculate that NK cells, γδT cells, or other cell types may defend patients with $CD4^+$ T-lymphocytopenia, we do not have direct evidence to corroborate our hypothesis.

As discussed above, $CD4^+$ T-lymphocytopenia was found in three patients. One may ask whether these patients may just show a reduction in lymphocyte counts and $CD3^+$ T cell counts (Table I), resulting in $CD4^+$ T-lymphocytopenia. However, as to the normal values of lymphocyte count in children and young adults, it has been reported that normal lymphocyte counts are 2,800/mm^3 (range, 1,200–5,200) at age 16 yr and 2,500/mm^3 (range, 1,000–4,800) at age 21 yr [15]. Japanese children also show similar lymphocyte counts. Thus, absolute lymphocyte counts in these patients (2,450, 972, and 1,364/mm^3 in a 15-yr-old patient 1, 21-yr-old patient 2, and 18-yr-old patient 3, respectively) may be low but not exceedingly low when we take their age into consideration. The basis for low-normal lymphocyte counts in these patients remains to be elucidated. Unknown viral infections may be associated with the present cases, or a higher proportion of $CD4^+$ T cells are not circulating in the blood, but are located in lymphoid tissues for some reason. However, we do not have data on the activation markers and adhesion molecule expression of the $CD4^+$ T cells to discuss this issue. As to total T cells in these patients, it has been reported that normal values of $CD3^+$ T cell count range from 493 to 1,739/mm^3 in children [16]. Thus, the value of 598, 602, and 521/mm^3 of $CD3^+$ T cell count in patients 1, 2, and 3, respectively, may be low but not be extremely low. Collectively, we think that $CD4^+$ T cells were rather specifically reduced in these patients.

The number of patients we evaluated was too small to permit us to ascertain the true incidence of infectious (opportunistic) complications in long-term survivors with $CD4^+$ T-lymphocytopenia following intensive cancer chemotherapy. We need further follow-up of these patients.

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